Photodynamic Impact on the Epiphyseal Plates

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Abstract. This study was carried out to prove the possibility of inhibition of long bones epiphyseal plates activity with photodynamic impact. Comparative analysis of the Chlorin E6 accumulation with transcutaneous and intraperitoneal administration mode, carried out on 175 laboratory mice showed the drug accumulates selectively in the epiphyseal plates of long bones, regardless of the mode of administration. 15 mice (males and females) at the age of active grownig were subjected to the single laser radiation impact on the knee joints area: 5 ones with transcutaneous Chlorine E6 administration, another 5 ones with intraperitoneal administration and the rest 5 without the drug. Histological samples of 15 experimental mice epiphyseal plates were examined by light microscopy, compared with 10 intact control mice. Influence of the laser radiation without administration of Chlorin E6 leads to intracellular swelling of epiphyseal plates chondrocytes. Influence of the laser radiation after transcutaneous or intraperitoneal injection of Chlorine E6 reduces significantly the total number of epiphyseal plates chondrocytes, without reducing the proportion of terminally-differentiated chondrocytes. Thus, the photodynamic impact inhibits the activity of epiphyseal plates of the mice.

Keywords. Photoditazin®, laser, chondrocytes, photosensitizer, growth.

Introduction

The method of photodynamic therapy (PDT) is based on the combination of laser irradiation (LI) and substances called photosensitizers (PS). Photosensitizers can accumulate selectively in intensively proliferating tissues and have selective sensitivity to certain light wavelengths of the optical range [9]. The absorption of light quanta of PS molecules in the presence of oxygen leads to a photochemical reaction, resulting in a triplet molecular oxygen O_2 ($X^3\Sigma_g^-$) transformation into a singlet $O_2(a^1\Delta_g)$, as well as a large amount of highly active radicals arising. Singlet oxygen and radicals cause to necrosis and apoptosis of target cells.

PDT is able to supress tissues proliferating. It is used in treatment of cancer [10], as well as many other diseases, including juvenile arthritis and degenerative diseases in children and adolescents [1, 3, 4, 8, 13]. We have the evidence of mark antibacterial and virostatic activity of PDT [2, 6, 7, 11].

However, the effects of photodynamic impact (PDI) on the epiphyseal plates of bones (which are tissues with high proliferative activity) haven't been investigated yet. This research is devoted to the studying of this aspect. It was realized in the experimental laboratory of The Russian Scientific Research Institute of Traumatology and Orthopedics named after R.R. Vreden.

1. Materials and Methods

1.1. The rate and depth of the photosensitizer penetration

Five groups of white nonlinear outbred mice at the age of active growing (2-6 weeks) were used to study the rate and depth of PS "Photoditazin®" (N-dimethylglucamine salt of Chlorine E6) penetration. There were 35 ones in each age group. 30 mice were experimental and 5 mice were control. In each control group the waxing hair (after euthanasia with an overdose of narcotic analgesics) and layer by layer dissection of tissue in the knee joint area were carried out. At each level (skin, joint capsule, hyaline cartilage, epiphyseal plate) intrinsic fluorescence of tissue was determined. The study was carried out with using the fluorescence spectroscopic diagnostic device "SPEKTR-Klaster" (LLC "Klaster" of the Institute of General Physics, RAS, Moscow) [5, 12]. The data were taken as the absolute unit.

Experimental animals were divided into two groups depending on the administration mode of the PS (transcutaneously as 0.5% gel-penetrator or intraperitoneally as 0.028% solution at a dose of 0.7 mg/kg). Each group was ivided into 3 more groups, according to the exposure time (for transcutaneous administration: I - 60 minutes, II - 90 minutes, III - 120 minutes; for intraperitoneal administration: I - 30 minutes, II - 60 minutes, III - 90 minutes). Euthanasia (by overdose of narcotic analgesic) was carried out for each group of animals reached the exposure time. After that the removal of residual drug from the skin, layer by layer dissection of tissues of both knee joints and the determination of the fluorescence of at least 5 measurements at each level (the skin, the joint capsule, hyaline cartilage, epiphyseal plate) were carried out. The highest and the lowest values were not taken, but the rest three ones were averaged and expressed in arbitrary units compared with control values.

1.2. Investigation of the photodynamic impact on the epiphyseal plates

15 white nonlinear outbred mice (males and females) at the age of active growing (36-64 days) were divided into three experimental groups of 5 animals. The knee joints of all the animals at the age 36 days were subjected to the single exposure of LI with wavelength (λ) = 662 nm, power = 1 W at a dose of 70 J/cm^2 during 1 min. 20 seconds from the 5 cm distance using the device for laser therapy "Atkus-2". The first group did not receive PS. The other animals received PS "Photoditazin®" at the dose of 0.7 mg/kg in 15 min. before experiment. The second group of animals got PS "Photoditazin®" transcutaneously as 0.5% gel-penetrator on the knee joints area. The third group of animals got PS "Photoditazin®" intraperitoneally as 0.028% solution.

One by one from each experimental group, animals were consistently derived from the experiment by overdose of narcotic analgesics in 3, 7, 14, 21 and 28 days after exposure time.

The control group was consisted of intact 10 white non-linear outbred mice (males and females). They were euthanized by overdose of narcotic analgesics: 2 individuals aged 36 and 40 days, and by one individual at the age of 44, 50, 51, 58, 65 and 180 days.

After the experiment, the histologic samples (both hind limbs from hip to ankle) of laboratory animals for examining were taken with subsequent fixation by 10% neutral buffered formalin. Thus, 20 macrosamples had been received and investigated in the control group and 30 macrosamples in the experimental groups (by 10 each).

The test material was subjected to dehydration, clearing, infiltration and embedding according to accepted methodics. 8-12 slice of 10 μm were prepared from each macrosample by using the sledge microtome MS-2. Then they were processed by subsequent Haematoxylin-eosin stain and conserved by Canada balsam.

Microscopic examination of the epiphyseal plates areas of the distal part of femur and the proximal part of tibia and counting the number of their constituent cells were carried out by using a binocular light microscope Mikromed-1 with magnification 900x.The results of calculation were subjected to correlation analysis.

2. Results

While the transcutaneous administration of PS, the maximum accumulation of the drug was noticed after exposure to 120 min.: in the skin - 21.79 units, in the knee joint capsule - 9.69 units, in hyaline cartilage - 8.89 units and in the epiphyseal plates - 6.48 units.

While the intraperitoneal administration of PS, the maximum accumulation of the drug was observed after exposure to 90 min.: un the skin - 14.42 units, in the capsule of knee joint - 6,65 units, in the hyaline cartilage - 7.66 units and in the epiphyseal plates - 7.47 units.

During the researching of distal femur (FB) and the proximal tibia (TB) epiphyseal plates in the first experimental group exposed to LI without PS, the increasing of epiphyseal plates thickness with promotion in the proportion of terminal and differentiated chondrocytes was noticed. The thickness of epiphyseal plates decreased to subnormal values in 21-28 days after the influence and the proportion of terminally differentiated chondrocytes was reduced but remaining higher than in the control group. These changes are presented in Tables 1 and 2.

Table 1. Changes in the thickness of epiphyseal plates after exposure to LI (hereafter the number of counted epiphyseal plates shown in parentheses) in comparison with the control group

Thickness of epiphyseal plates	Bones	Control group	Experimental group I		Reliability of changes
			Time after exposure to LI		
			3-7 days	21-28 days	
Expressed in μm	FB (10)	178.5±37.4	209.1 ± 25.5	164.9 ± 15.3	P > 0.05
	TB (10)	183.6±22.1	283.9 ± 47.6	190.4 ± 13.6	P > 0.05
Number of cells	FB (10)	23.3±1.1	17.0 ± 0.9	14.5 ± 1.7	P < 0.05
	TB (10)	21.7±1.6	22.5 ± 1.8	15.5 ± 0.9	P < 0.05

Table 2. Changes in the relative share (%) of terminally differentiated chondrocytes of epiphyseal plates after the action of LI in comparison with the control group

Thickness of epiphyseal plates	Bones	Control group	Experimental group I		Reliability of changes
			Time after exposure to LI		
			3-7 days	21-28 days	
Expressed in μm	FB (10)	50.9±3.1	72.3 ± 2.2	58.9 ± 4.5	P < 0.05
	TB (10)	41.5±3.8	75.2 ± 4.6	59.2 ± 4.2	P < 0.05
Number of cells	FB (10)	31.8±2.3	51.2 ± 2.1	38.2 ± 1.2	P < 0.05
	TB (10)	29.1±1.9	46.0 ± 2.9	43.1 ± 2.5	P < 0.05

In the second and third experimental groups, that were exposed to LI with transcutaneous and intraperitoneal administration of PS, decrease of epiphyseal plates thickness and reducing of the total chondrocytes number in FB distal and TB proximal epiphyseal plates were found. At the same time, the transcutaneous administration of PS caused the increase of terminally differentiated chondrocytes amount while the intraperitoneal administration of PS did not have a significant impact on them. These changes are presented in Tables 3 and 4.

Table 3. The average thickness of the epiphyseal plates

Thickness of epiphyseal plates	Bones	Control group	Experimental groups		
			I	II	III
Expressed in μm	FB	178.5±37.4	192.1±15.3 (10)	168.3±13.6	159.8±30.6
	TB	183.6±22.1	214.6±20.4 (10)	176.8±11.9	144.5±15.3
Number of cells	FB	23.3±1.3 (7)	16.1±1.2 (10)	16.2±1.4 (5)	11.7±1.4 (5)
	TB	21.7±1.6 (8)	20.4±1.5 (10)	15.3±2.1 (6)	13.8±2.3 (5)

Table 4. Changes in the relative share (%) of terminally differentiated chondrocytes, in the epiphyseal plates

Thickness of epiphyseal plates	Bones	Control group	Experimental groups		
			I	II	III
Expressed in μm	FB	50.9±3.1	67.5±3.3 (10)	48.3±7.9	47.1±4.1
	TB	41.5±3.8	68.0±5.0 (10)	47.0±2.0	46.3±1.9
Number of cells	FB	31.8±2.3 (7)	47.3±2.5 (10)	28.5±1.9 (5)	48.7±2.9 (5)
	TB	29.1±2.8 (8)	45.2±1.6 (10)	34.8±1.9 (6)	34.3±2.8 (5)

3. Conclusions

In spite of administration modes (transcutaneous or intraperitoneal) PS accumulates in the bone epiphyseal plates selectively.

The impact of LI on the epiphyseal plates leads to the increasing of its thickness, that can be explained by swelling of the chondrocytes cytoplasm.

In spite of administration modes, PS causes the epiphyseal plates decreasing and reducing of the chondrocytes total number in them.

PDI with intraperitoneal administration of PS does not cause the increasing of terminally differentiated chondrocytes share. Taking into account the reducing number of chondrocytes in epiphyseal plates, it can indicate only a general inhibition of the differentiative and regenerative potential of epiphyseal plates chondrocytes.

Thus, PDI inhibits the epiphyseal plates activity of long bones in experimental animals. The absence of terminally differentiated chondrocytes share reducing suggests that this inhibition is reversible.

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